An Approach to the Development of Quantitative Models to Assess the Effects of Exposure to Environmentally Relevant Levels of Endocrine Disruptors on Homeostasis in Adults

Nira Ben-Jonathan,[1] Ralph L. Cooper,[2] Paul Foster,[3] Claude L. Hughes,[4] Patricia B. Hoyer,[5] Diane Klotz,[6] Michael Kohn,[6] Dolores J. Lamb,[7] and George M. Stancel[8]

[1]Department of Cell Biology, University of Cincinnati, Cincinnati, Ohio USA; [2]National Health Effects and Environmental Research Laboratories, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina USA; [3]Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina USA; [4]Center for Women's Health, Cedars-Sinai Medical Center, Los Angeles, California USA; [5]Department of Physiology, University of Arizona Health Science Center, Tucson, Arizona USA; [6]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina USA; [7]Department of Urology, Baylor College of Medicine, Houston, Texas USA; [8]Department of Integrative Biology, Pharmacology, and Physiology, University of Texas Medical School, Houston, Texas USA

The workshop "Characterizing the Effects of Endocrine Disruptors on Human Health at Environmental Exposure Levels" was held to provide a forum for discussions and recommendations of methods and data needed to improve risk assessments of endocrine disruptors. This article was produced by a working group charged with determining the basic mechanistic information that should be considered when designing models to quantitatively assess potential risks of environmental endocrine disruptors in adults. To reach this goal, we initially identified a set of potential organ system toxicities in males and females on the basis of known and/or suspected effects of endocrine disruptors on estrogen, androgen, and thryoid hormone systems. We used this integrated, systems-level approach because endocrine disruptors have the potential to exert toxicities at many levels and by many molecular mechanisms. Because a detailed analysis of all these untoward effects was beyond the scope of this workshop, we selected the specific end point of testicular function for a more detailed analysis. The goal was to identify the information required to develop a quantitative model(s) of the effects of endocrine disruptors on this system while focusing on spermatogenesis, sperm characteristics, and testicular steroidogenesis as specific markers. Testicular function was selected because it is a prototypical integrated end point that can be affected adversely by individual endocrine disruptors or chemical mixtures acting at one specific site or at multiple sites. Our specific objective was to gather the information needed to develop models in the adult organism containing functional homeostatic mechanisms, and for this reason we did not consider possible developmental toxicities. Homeostatic mechanisms have the potential to ameliorate or lessen the effects of endocrine disruptors, but these pathways are also potential target sites for the actions of these chemicals. *Key words*: androgen, estrogens, endocrine disruptors, homeostasis, spermatogenesis, testicular steroidogenesis, thyroid hormones. — *Environ Health Perspect* 107(suppl 4):605–611 (1999).
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The endocrine system of adult animals is equipped with a set of mechanisms that regulate circulating levels of endogenous hormones. For example, a series of feedback loops involving the hypothalamus, pituitary, and the gonads, adrenals, and thyroid gland regulates the synthesis of sex steroids, glucocorticoids, and thyroid hormones, respectively. At the same time, elimination of these hormones via biotransformation is catalyzed by enzymes in the liver and other sites that are inducible by hormones themselves as well as other agents (*1,2*). Thus, the adult mammalian organism has several homeostatic mechanisms that maintain the levels of endogenous estrogens, androgens, and thyroid hormones within certain ranges.

These same homeostatic mechanisms also can dampen or moderate the effects of endocrine disruptors that affect estrogenic, androgenic, or thyroid hormone systems. In addition it is well established that many acute effects of abnormally high or low levels of endogenous hormones are reversible, e.g., normal menstrual cycles resume when women discontinue using contraceptives (*2*), and it is reasonable to expect that the same might be true of acute effects of endocrine disruptors. This is in contrast to the developing organism in which feedback mechanisms controlling hormone synthesis and elimination mechanisms controlling hormone degradation may not be fully functional and in which early developmental exposures may lead to irreversible changes. Hence, it seems appropriate to discuss potential effects of endocrine disruptors in the adult separately from those in the developing organism, and this article focuses on the former.

A workshop on characterizing the effects of endocrine disruptors on human health at environmental exposure levels was held to provide a forum for discussions and recommendations of methods and data needed to improve risk assessments of endocrine disruptors. The authors of this article were members of a working group that addressed homeostasis and endocrine function in adults and how perturbations of endocrine homeostasis may lead to disease. The charge to this working group was to determine the basic mechanistic information that should be considered when designing models to quantitatively assess the risks of endocrine disruptors present at environmentally relevant levels. The specific focus was on endocrine disruptors that affect estrogen, androgen, and thyroid hormone systems, but in the broadest conceptual sense. To some extent, the required mechanistic information depends upon the specific end point one wishes to model, and during a 2-day meeting it was clearly impossible to develop a comprehensive list of information required to analyze all possible adverse effects of every potential endocrine disruptor. Therefore, our approach was to consider a variety of potential untoward effects on the basis of our collective knowledge of endocrine mechanisms, to select one we felt was amenable to development of a risk assessment model, and to delineate the information necessary to construct a model. This would provide a starting point for analysis of one end point, but more important would illustrate the general considerations that basic endocrinologists would incorporate into a model of endocrine disruptor actions. Many of these considerations would likely

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The contents of this manuscript reflect the opinions of the authors, who were members of a working group on Homeostasis and Endocrine Function in Adults and were neither approved nor disapproved by those in attendance at the workshop. Also note that the series of questions considered at the workshop and in this manuscript was developed by the organizing committee rather than the members of the working group.

Address correspondence to G.M. Stancel, Dept. of Integrative Biology, Pharmacology, and Physiology, University of Texas Medical School, PO Box 20708, Houston, TX 77225. Telephone: (713) 500-9880. Fax: (713) 500-0988. E-mail: gstancel@gsbs.gs.uth.tmc.edu

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pertain to the effects of endocrine disruptors on other systems and thus are likely to have some generalized applicability.

Once a model system was selected, the goal was to answer questions originally posed by the organizing committee, which the working group also thought were important to consider. These are as follows:

- Are there adequate/relevant animal models for evaluating potential human effects of low-dose exposure to endocrine disruptors?
- What should be included in baseline models to adequately describe quantitative relationships among the processes maintaining homeostasis?
- How do perturbations in this balance lead to disease, e.g., impaired reproductive function, cancer?
- How can these changes be quantified?
- By what mechanisms do endocrine disruptors perturb endocrine homeostasis and function and alter risks from normal levels of endogenous hormones?
- How do differences in lifestyle factors (e.g., diet, nutritional status, physical activity) affect sensitivity to endocrine disruptors?
- How does the age of the endocrine system alter susceptibility to endocrine disruptors?

Overview of Endocrine Disruptor Action

The biologic actions of endocrine disruptors are produced by the same general mechanisms as those of other chemicals including hormones, drugs, or other toxicants. The action of all chemicals on the body may be divided by dichotomy into two major steps. The first is the series of interactions between the portal of entry of the endocrine disruptor and its concentration at tissue sites. These interactions involve absorption, distribution (e.g., binding to plasma proteins, sequestration in tissue sites), and elimination of the endocrine disruptor. The second set of interactions dictates the magnitude of the effect the endocrine disruptor produces at various tissue sites. These interactions involve the entire series of cellular events between the initial interaction of the endocrine disruptor at receptors (or nonreceptor sites) and the ultimate biologic response of the target cell. This simplified schema is illustrated in Figure 1.

Pharmacokinetic Issues

The conceptual framework required to describe the relationships between the amount of an endocrine disruptor that enters the body at a portal of entry and its concentration at tissue sites is already available. However, several specific research needs must be met if we are actually to obtain reliable quantitative measures of these parameters. These include the following: *a*) The analytic capability must be available to measure amounts of endocrine disruptors in the body after exposure to environmentally relevant levels. In most cases the chemical or immunologic methods and reagents used to make these measurements are unavailable. *b*) In the same vein, there is often no reliable and affordable source from which investigators may obtain high-purity endocrine disruptors. Compounds of verified purity are essential to unequivocally assign biologic actions to parent compounds (rather than impurities) and to provide standards for analysis of compound levels in biologic samples and the environment. *c*) Another important need is for appropriate radioactively labeled endocrine disruptors to accurately determine pharmacokinetic parameters, levels of specific metabolites in bodily fluids and tissues, and receptor-binding properties. This may be especially critical for identifying products formed by biotransformation of endocrine disruptors that may have greater, lesser, or qualitatively different biologic activity than the parent compound. If these needs can be met, determining the important pharmacokinetic parameters for any endocrine disruptor becomes technically feasible. In the absence of experimental data at low exposures, a physiologically realistic model that reproduces effects measured at higher doses can be used to extrapolate to environmentally relevant levels.

Endocrine Disruptor Actions at Target Organ Sites

The conceptually more challenging issue is to develop quantitative models based on biologic mechanisms that can be used to predict adverse effects of endocrine disruptors at various tissue sites after low-dose exposures. This requires a knowledge of the concentration of endocrine disruptor at the target site, the nature of the effect(s) produced in the tissue, a determination of what degree of change constitutes an adverse effect, the basic mechanism(s) involved, and the nature of the dose–response relationship for the endocrine disruptor. If the endocrine disruptor alters the levels of endogenous hormones or any of their biologically relevant metabolites, then the dose–response relationships for these molecules is also required. Similarly, if the toxicant alters the expression of proteins regulated by the hormone receptor, measurement of those responses is required.

Given this perspective, we initially developed a list of target organ sites in both

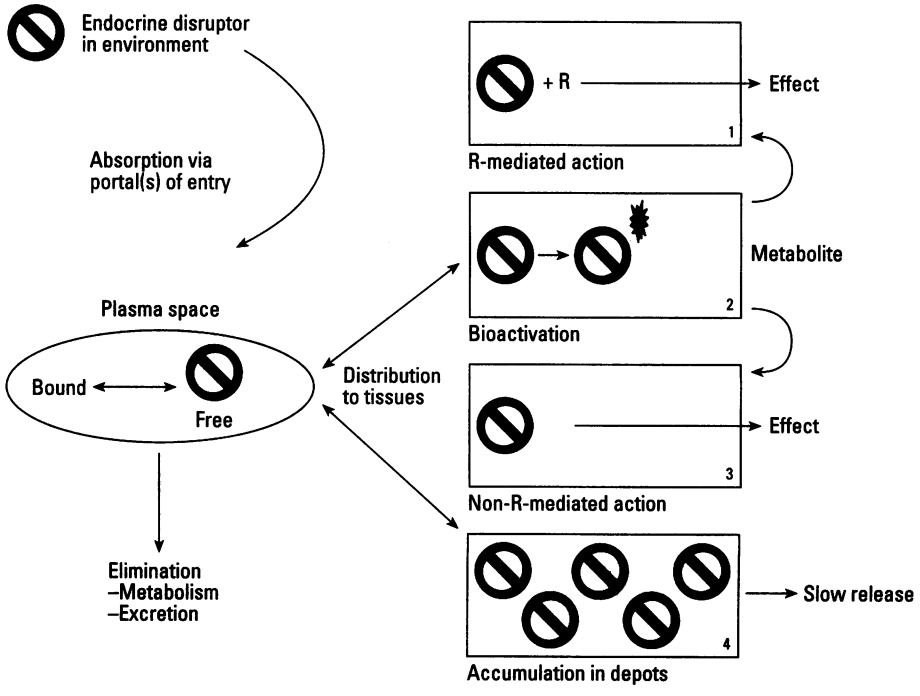

Figure 1. Overview of endocrine disruptor action. An endocrine disruptor (ED) present in the environment (upper left corner) enters the body at one or more portals of entry and is absorbed into the plasma compartment, where it may exist free or bound to plasma proteins. The ED may be eliminated (bottom left) by metabolism to an inactive compound and/or excretion via the urine or other routes. The ED distributes to tissue compartments (right panels). At site 1 the endocrine disruptor produces an effect following binding to a hormone receptor (R), and at site 3 the ED produces an effect via a nonreceptor mechanism. At site 2 an ED with little or no intrinsic activity may undergo bioactivation to yield a metabolite(s) that may disrupt the endocrine system via receptor (site 1) or nonreceptor (site 3) mechanisms. The ED may also accumulate in depot sites such as adipose tissue (site 4) and undergo slow release over prolonged periods of time.

the male and female at which potential endocrine disruptors affecting estrogen, androgen, or thyroid hormone systems should be considered. This listing does not imply that such actions are established or even likely. Rather, the objective is to develop a list of sites and effects that a knowledgeable endocrinologist would examine to determine if a chemical were acting as an endocrine disruptor. A listing of such sites is given in Tables 1 and 2 for the female and male, respectively. In addition, potential adverse effects of endocrine disruptors at each site are also given. In many cases, effects at multiple sites and involving multiple mechanisms are listed as having the potential to produce the same effect. For example, a hormonelike effect could be produced directly by an endocrine disruptor with agonist activity occupying a receptor site in the tissue in question. Alternatively, the same action at a tissue site could be produced by an endocrine disruptor acting on the hypothalamic–pituitary–gonadal axis to increase endogenous hormone production, or by actions at the liver to decrease elimination of endogenous hormones, etc.

Because of the large number of potential effects, each was further ranked as being of high, medium, or low concern. This ranking of effects is admittedly subjective, but it represents a consensus (unanimity in almost all cases) of the working group on the basis of the following criteria: *a*) the likelihood that the effect would have a substantive impact on reproduction and hence survival of the species; *b*) the likelihood that the effect would be potentially life threatening to an affected individual; *c*) the impact of the effect on the quality of life of an individual; and *d*) the estimated likelihood that environmentally relevant levels of endocrine disruptors are sufficient to actually produce the effect. These criteria were not weighted quantitatively in the ranking process but were considered in toto by members of the working group in reaching their individual levels of concern.

To further focus potential future studies, the working group selected a smaller subset of potential effects from Tables 1 and 2. This selection was performed on the basis of perceived importance for human health and the potential likelihood that quantitative models of the effect could be developed that would be useful for predicting low-dose effects in the human population. In most cases, the effect selected is actually an aggregate response expected to result from the action of an endocrine disruptor at multiple sites. The effects selected were testicular function, endometrial cancer, the female hypothalamic–pituitary–gonadal axis up to and including successful implantation, reproductive senescence in the female, mammary cancer, and adverse effects on the cardiovascular system and bone. Testicular function was subsequently chosen as the effect to consider as a prototype for modeling studies and is discussed at length in the last section of this article. A brief rationale for considering each of the other six effects follows and is presented as a possible stimulus for modeling studies focusing on these end points.

Table 1. Potential sites of endocrine disruptor effects in the adult female and priority level of concern.

Site	Priority concern of effect		
	High	Medium	Low
Uterus	Infertility Tumors	Menstrual bleeding Endometriosis	Infection
Oviduct			Infertility
Vagina			Vaginitis
Mammary gland	Cancer Lactation		Fibrocystic disease
Ovary	Ovulation Oocyte number Cancer Steroidogenesis		Luteal phase defects Cysts
Hypothalamus and pituitary	Altered cyclicity Ovulatory failure Reproductive senescence Tumors Hormone secretion	Onset of puberty Sexual behavior	
Brain	Stroke	Alzheimer's disease Dementia Depression	
Thyroid	Cancer	Hormone secretion Goiter	C-cell tumors
Bone	Osteoporosis		
Cardiovascular system	Hypertension Coronary artery disease Venous thromboembolic disease		
Gastrointestinal tract		Colon cancer Ca^{2+} absorption Bioactivation (of endocrine disruptors)	Hormone clearance Ion transport and fluid secretion Mobility
Adrenal		Steroidogenesis	
Adipose tissue	Accumulation of endocrine disruptors	Metabolism of endocrine disruptors and hormones	
Immune system		Autoimmune disease Immunocompetence	
Liver	Hormone metabolism Tumors	Serum-binding protein synthesis Biliary function	Lipoprotein synthesis Clotting factor synthesis
Skin			Acne Hirsutism Hormone metabolism Baldness Atrophy
Bladder			Incontinence

Endometrial Cancer

Endometrial cancer is a major human disease that affects tens of thousands of women in the United States each year. Epidemiologic studies have firmly established that exposure to exogenous estrogens increases the risk of endometrial cancer in postmenopausal women receiving hormone replacement therapy (*3*), and much evidence suggests that total lifetime exposure to endogenous estrogens is a major risk factor for this disease and other human cancers (*4*). The rodent uterus has been one of the most extensively studied *in vivo* models of endocrine-disruptor action, and there are several very plausible molecular mechanisms by which exposure to environmental estrogens would be expected to alter the incidence of this disease. In addition, the endometrium is one of the most sensitive estrogen-responsive tissues, and endometrial biopsies are potentially available for the study of human exposures and their sequelae.

Table 2. Potential sites of endocrine disruptor effects in the adult male and priority level of concern.

Site	Priority concern of effect		
	High	Medium	Low
Testis	Spermatogenesis Tumors Peptide hormone synthesis Steroidogenesis		Varicocele
Epididymis	Sperm maturation Sperm transport		Obstruction Infection
Prostate	Tumors Benign prostatic hyperplasia	Fluid composition	Prostatitis (various) Obstruction
Seminal vesicle		Fluid composition	
Ejaculatory duct			Cysts
Penis		Erectile dysfunction	Ejaculatory dysfunction
Mammary gland		Cancer Gynecomastia	
Hypothalamus and pituitary	Tumors Hormone secretion	Puberty onset Sexual behavior	Reproductive senescence
Brain		Stroke	Aggression
Thyroid	Cancer	Hormone secretion Goiter	C-cell tumors
Bone			Osteoporosis Bone maturation
Cardiovascular system	Coronary artery disease Hypertension Venous thromboembolism		
Gastrointestinal tract			Motility Hormone clearance
Adrenal		Steroidogenesis	
Skeletal muscle			Hypertrophy Atrophy
Skin and hair			Acne Baldness Hormone metabolism
Adipose tissue	Hormone metabolism Endocrine disruptor accumulation		Hormone distribution
Kidney			Androgen-regulated proteins
Immune system			Antibody secretion into genital tract

The female hypothalamic–pituitary–gonadal axis—up to and including successful implantation. There is a wealth of information describing the physiologic interplay of the brain–pituitary–ovarian axis, the regulation of follicular development, ovulation, and implantation in both the rat and human. For many of these processes, homologous mechanisms have been identified for the rodent and human. Furthermore, this endocrine axis has been the focus of numerous toxicologic studies identifying both the target tissue(s) and cellular mechanisms involved in adverse changes in the female's reproductive capacity. In rodent models, environmental agents target the central nervous system and disrupt the hypothalamic control of anterior pituitary leutinizing hormones (LH) and prolactin secretion (both xenoestrogens and non-estrogenic neuroactive compounds) (*5–7*), the pituitary itself (i.e., metals, xenoestrogens) (*8*), the ovary (e.g., loss of follicles, disruption of ovulation, and modified corpus luteum function), and ova transport and implantation (e.g., agents that disrupt prolactin and progesterone availability) (*9,10*). In many cases dose–response data on the toxicant are available.

Reproductive Senescence in the Female

Female reproductive senescence (menopause) results from ovarian depletion of oocytes (primordial follicles) that are available to develop for ovulation. Environmental chemicals that extensively destroy primordial follicles can lead to premature menopause because the pool of primordial follicles is nonregenerating (*11*). Menopause is associated with a variety of health problems, including osteoporosis, cardiovascular disease, arthritis, urinary tract infections, depression, and an increased risk of ovarian cancer. Therefore, as a woman ages, her overall health is significantly affected by the onset of menopause, which can be further impacted by environmental factors. Several classes of environmental chemicals, chemotherapeutic agents, occupational epoxides, and polycyclic aromatic hydrocarbons (e.g., compounds contained in cigarette smoke) destroy primordial follicles in laboratory animals (*11*). Epidemiologic evidence indicates that these agents also shorten the reproductive life span of women (*12*). For example, women smokers are known to enter menopause 2–6 years earlier than nonsmokers. Therefore, chemicals that cause premature ovarian failure can be classified as indirect endocrine disruptors because they destroy the source of steroid hormones, which interferes with their target organs and disrupts the balanced pattern of neuroendocrine hormone feedbacks.

Because the adverse effects of low-dose exposure to reproductive toxicants may be more prominent after chronic exposure, effects such as reproductive senescence in the female (i.e., menopause in humans, reproductive life span in rodents and humans) that occur gradually over time may be especially important to evaluate. As noted above, there are a number of studies suggesting that environmental agents may accelerate the loss of ovarian follicles in humans. One concern for this parameter is the lack of an appropriate animal model. However, identifying an accelerated loss of ovarian follicles in response to low-dose exposure in the rat could provide useful information for identifying potential human toxicants. Reproductive aging in the female rodent has been studied extensively and as the final disruption of reproductive capacity in the female represents the cumulative effects of events occurring throughout the animal's life span, this measure could be particularly sensitive to low-dose exposures.

Mammary Cancer

Breast cancer is the most common endocrine-related malignancy among women, with an estimated 180,000 new cases diagnosed each year in the United States alone. Ultimately, 1 of 10 women will develop breast cancer during her lifetime. Development of the normal breast, carcinogenesis, and the progression of breast cancer are regulated by hormones and growth factors, among which the effects of estrogens are especially prominent and best understood at the molecular level. Studies using both mammary tumors in rodents and human breast cancer cells in culture have established that estrogens affect

mammary epithelial cell proliferation and differentiation, induce growth factor production, and alter the expression of proteolytic enzymes and cell adhesive molecules (*13,14*). Breast cancer cells express a diversity of normal and mutated estrogen receptor transcripts which, if translated into functional proteins, could produce receptors with different affinities for natural and synthetic estrogens (*15*). In addition, selected estrogen metabolites can lead to the formation of covalent DNA adducts and trigger damage (*16*). The possibility that prolonged exposure to endocrine disruptors has an adverse effect on the development of breast cancer requires careful consideration and analysis.

Adverse cardiovascular and skeletal actions. Estrogens protect against atherosclerosis and myocardial infarcts in premenopausal women. The cardiovascular protection of estrogens is mediated indirectly by effects on lipoprotein metabolism, and more recent studies have indicated these hormones may have direct protective actions on vascular smooth muscle and endothelium (*17*). Osteoporosis, which primarily affects postmenopausal women, represents a progressive bone loss, reflecting a shift in the balance between cells responsible for bone formation and resorption. In studies using rodents, estrogens inhibit bone loss at relatively low concentrations (*18*). Interestingly, both vascular and bone cells have recently been reported to express the newly discovered estrogen receptor β and to have a higher binding affinity for several environmental estrogens (e.g., genistein, coumestrol) than the classical estrogen receptor α (*19–21*). Cardiovascular disease is the leading cause of death in women and fractures in elderly women are a major cause of morbidity and mortality. It thus seems particularly important to elucidate the potential effects of endocrine disruptors, both detrimental and beneficial, on these conditions.

Conceptual Approach to Develop a Model to Analyze Low-Dose Effects of Endocrine Disruptors on Testicular Function

The general process we selected as a prototype for modeling purposes is testicular function, and within that overall process there are three specific functional end points we chose to consider, spermatogenesis, sperm characteristics, and steroidogenesis. Testicular function was selected for several major reasons: *a*) these are significant biologic end points for the health and quality of life of the individual as well as the survival of the species, *b*) it should be possible in some cases to obtain human as well as animal data that can be used to corroborate the models one develops, and *c*) collectively these end points provide an integrated readout of a coordinated biologic system that is subject to endocrine disruption by many toxicants acting at a number of sites and by a variety of mechanisms. This last point might be particularly important for several reasons when considering endocrine disruptors. First, a single endocrine disruptor might affect the male reproductive system at multiple sites, and hence an effect at one of the cumulative end points noted above might occur at a lower dose than an effect at a single target site studied individually. For example, a relatively small fractional inhibition of several steps in a pathway may lead to a more dramatic quantitative change in the overall end point of the pathway. Second, an end point such as testicular function may be used to study the effects of mixtures of endocrine disruptors and other toxicants that may act at different sites and by different mechanisms, all of which would affect net testicular function.

Are There Adequate/Relevant Animal Models for Evaluating Potential Human Effects?

One of the attractive features of developing a model to analyze the effects of endocrine disruptors on testicular function is that a number of good animal models are available. Baboons and Old World monkeys are particularly good models for the study of spermatogenesis and sperm characteristics. Certain aspects of these processes can also be studied in rodent models that would be relevant to humans, and rodent models are particularly useful for certain laboratory-based experimental studies. In addition, domestic farm animals are also available, and in many cases extensive data on these parameters are available from historical breeding records. The enzymatic pathways involved in testicular steroidogenesis are essentially identical in humans and most other mammals, the structure and regulation of the enzymes from different species are generally quite similar, and there is a large amount of available literature on these pathways, hormone levels, etc., in humans and animal species. As previously noted, another important consideration is that human data can be obtained in many cases to validate animal-based models. It should also be noted that the sensitivity of different species to an endocrine disruptor may vary dramatically. The availability of a number of animal models provides the opportunity to study those with sensitivities similar to humans or at least to study sensitive animal species in cases for which human sensitivity is not yet established.

What Should Be Included in Baseline Models to Adequately Describe Quantitative Relationships among the Processes Maintaining Homeostasis?

The major information included in models falls into one of several categories. One major area is spermatogenesis and daily sperm production rates are a major parameter to be incorporated. In conjunction with a measure of the quantity of sperm produced, standard measures of sperm characteristics must also be included, e.g., sperm motility and morphology, sperm penetration assay, cervical mucus penetration.

Substantial information on hormone levels should also be included in models of testicular function. Serum levels of the peptide hormones LH, follicle-stimulating hormone, prolactin, and inhibin should be provided, as well as free and protein-bound levels of numerous steroids (testosterone, dihydrotestosterone, androstenedione, estradiol, 17-hydroxyprogesterone). The actual testicular levels of steroids should also be measured, as endocrine disruptors could conceivably affect the two pools of steroid hormones differentially. Sample collection should be performed at multiple times to account for pulsatile hormone secretion, circadian rhythms, and seasonal variations, and these should be incorporated into models. Also, the rates of production of these hormones under various conditions and their rates of degradation are required for a baseline model as well as their binding affinities for plasma and cellular proteins.

In addition to these biochemical measures, morphometric studies should be performed to measure the number of cells present for all major cell types in the testes, to assess the number and nature of cell–cell associations; and mitosis and meiosis (the number and nature of cells at various stages of spermatogenesis).

Although it might be difficult to incorporate information on fertility into quantitative models of risk assessment, it would be important to obtain information on this parameter to see if changes and trends in this parameter appear consistent with the more readily quantifiable measures above. Data on male fertility in both humans and domestic animals should be collected to the degree they are available, but caution must be exercised in interpretation of results. For example, animal breeders are likely to bias this end point by intentionally selecting for high fertility in males. Also, in the case of humans some standard indices of male fertility (e.g., number of offspring) may be difficult to interpret because they are heavily influenced by multiple factors (such as lifestyle choices or use of contraceptives). Thus it may be advantageous to consider additional end points in humans, e.g., the

time to conception for couples wishing to have children.

How Do Perturbations in the Homeostatic Balance Caused by Endocrine Disruptors Lead to Untoward Effects Such As Impaired Reproductive Function?

The working group considered this question from both qualitative and quantitative perspectives. From a qualitative perspective we considered what biologic processes likely to be affected by endocrine disruptors should be considered in the development of models. From a quantitative perspective we then considered what magnitude of change constitutes an adverse effect or impaired function. Both of these questions should be considered because one might be able to detect quantitative changes in many parameters that would not necessarily constitute an adverse effect of an endocrine disruptor.

Qualitatively, the processes that should be incorporated into risk assessment models include synthesis of testicular hormones (both steroid and peptide); clearance of hormones; biotransformation of hormones to metabolites with quantitatively different efficacy or potency, or biologic activities qualitatively different than those of the parent compound; and the disposition of hormones, e.g., degree of plasma protein binding, volume of distribution, and sequestration in adipose tissue, bone, or other repository sites. In addition, models should incorporate basal levels of hormone receptors as well as their fractional occupancy by endogenous hormones and endocrine disruptors and resultant changes in function. It is important to emphasize that in some cases hormone receptors may have activity in the absence of ligand binding, and that receptors may be activated by mechanisms other than binding of their cognate hormones. For example, steroid hormone receptors may be activated by phosphorylation secondary to activation of growth factor-initiated kinase cascades (*22*).

In addition to considering levels of receptors and their ligands, it is important to consider the cellular context in which these molecules produce their effects. For example, the net cellular effect that results from the binding of a polypeptide hormone to a cell surface receptor may depend on the level of G proteins, enzymes involved in the formation and breakdown of second messengers, etc. (*23*). The effects of nuclear receptors may depend upon the levels of coactivators, corepressors, and other proteins involved in transcription control (*24*). The levels and activities of these types of factors must also be incorporated into quantitative models.

Finally, factors that affect either the rates of cell proliferation or cell death in the testes must also be included. These could be agents that act via mechanisms directly involving receptors for hormones and autocrine or paracrine factors as well as agents that act by virtue of their chemical or physical properties rather than receptor interactions. These latter agents might include toxicants considered historically under the rubric of reproductive toxicants but that have not previously been thought of as endocrine disruptors.

In terms of defining the magnitude of change that constitutes endocrine disruption, the working group essentially relied on the basic concept of homeostasis. If one views homeostasis as a process to maintain biologic parameters within a normal range, then any change that causes the value of a parameter to fall outside the normal set of values can be considered an adverse effect. In other words, homeostatic mechanisms could not correct for the biologic insult. This is also consistent with the manner in which one considers the values for any biologic parameter in an individual. The physician or the veterinarian knows that values for any parameter vary within the population, and thus he/she considers whether the measured parameter falls within the normal range. Thus the working group considers an adverse effect to be any change in the parameters of a model (e.g., hormone levels, receptor occupancy, steroid clearance) that falls outside the normal range for the species being considered. In many cases the range of normal values for parameters of interest and their periodic variation have already been determined for humans and many animal species.

How Can These Changes Be Quantified?

Most parameters previously identified can be quantitatively measured by existing chemical, immunologic, or bioassay methods. This is certainly true for the endogenous hormones and receptors that play central roles in testicular function. It is important to reemphasize, however, that this is not the case for many of the endocrine-disrupting chemicals in the environment and we recommend that efforts be made to develop such technologies and to make high purity chemicals available for both analytic and biologic studies.

Incorporating such data into a quantitative model also requires knowledge of the binding affinities of the xenobiotic agent to receptors and carrier proteins. Furthermore, the partitioning of the agent between blood and tissues and the kinetics of metabolic clearance of the toxicant must be measured.

By What Mechanisms Do Endocrine Disruptors Perturb Endocrine Homeostasis to Alter Testicular Function and Increase Risks from Normal Levels of Endogenous Hormones?

At the cellular level, endocrine disruptors may act directly on the testes or indirectly at other sites to affect testicular function, and in either case the molecular mechanism of these effects may be due to interactions with hormone receptors or at nonreceptor sites. Within the testes the major receptors to be considered are those for estrogens, androgens, and thyroid hormones, and endocrine disruptors could function as agonists or antagonists at these receptor sites. Agents acting at the level of the testes but by nonreceptor mechanisms include those that alter spermatogenesis, germ cell proliferation or apoptosis, and autocrine or paracrine interactions affecting tissue functions, meiosis, or differentiation of spermatids.

Receptor mechanisms at nontestes sites would again include endocrine disruptors with either agonist or antagonist activity at estrogen, androgen, and thyroid receptor sites. Models should put particular emphasis on these receptors in the hypothalamus and pituitary, which can affect the testes via alterations in gonadotropin production and secretion and by nuclear receptors in the liver that can alter hormone clearance via enzyme induction and endogenous hormone disposition via changes in the synthesis of serum-binding proteins such as sex steroid-binding globulin (*25*).

A variety of nonreceptor mechanisms at nontestes sites should also be included. These include any hepatic effects that would alter levels or activities of metabolic enzymes using endogenous hormones as substrates; any hypothalamic or pituitary effects that alter gonadotropin or prolactin levels (e.g., hypothalamic effects of compounds with dopaminergic activity that might alter gonadotropin-releasing hormone production), or mechanisms that perturb thyroid-stimulating hormone and/or thyroid-releasing hormone levels.

What Other Factors Should Be Considered in the Development of Models to Assess the Effects of Endocrine Disruptors on Testicular Function?

There are an enormous number of additional factors that could be built into models to assess low-dose effects of endocrine disruptors on testicular function or other biologic end points, and these could not be discussed in depth during a 2-day meeting. The working group thus decided to list factors likely to be of major importance. The major areas to be considered are age, disease, genetic variability, and lifestyle factors. There is substantial literature on many of these factors which would serve to provide specifics for incorporation into models.

There is substantial literature on the effects of age on testicular function in humans and animals. This literature includes information on the three general

end points, sperm production, sperm characteristics, and steroidogenesis, that would be the focus of an integrated model to describe testicular functioning in response to endocrine disruptors.

A large number of diseases affect testicular function either directly or indirectly. However, many of these are not necessarily related to environmental exposures to endocrine disruptors. There is a strong interest in the possible relationship(s) between endocrine disruptors and the increasing incidence of testicular cancer. The working group felt that this specific disease should be explicitly considered.

Experimental studies can be performed with inbred strains of animals to minimize the effects of genetic background, but genetic differences must be included in models designed to predict human health effects. Certainly ethnicity should be included in baseline models, as should genetic variations likely to have major effects on the levels of endocrine disruptors and/or endogenous hormones (e.g., bioactivation or clearance) or their effects (receptor subtypes). The recently announced National Institute of Environmental Health Sciences Environmental Genome Project can be expected to provide useful information about genetic loci of special importance for quantitative modeling purposes.

A variety of lifestyle factors are also of prime importance to a biologic end point such as testicular function, and the literature contains specific information on each of these that could be used for development of models. Specific factors include diet and nutritional status; lifestyle factors such as stress and exercise; geographic location (as temperature and photoperiods can affect testicular function); therapeutic and recreational drug use; and smoking and drinking patterns.

Summary

The use of an integrated biologic read-out such as testicular function is particularly well suited to develop models that can be used to quantitatively assess the effects of endocrine disruptors in adult organisms. This approach is initially more complex than those that focus on more discrete end points such as a single type of receptor-mediated response (e.g., the activation of an individual gene or reporter by an environmental estrogen or androgen). Although the latter can be very valuable for many purposes (e.g., high throughput screening), the use of integrated system end points enables one to model effects at the level of the whole organism. More specifically, an integrated approach *a*) can incorporate homeostatic mechanisms that cannot be quantitatively incorporated into more discrete end points measured in a single cell type or organ; *b*) can describe cases in which a single endocrine disruptor may have effects at multiple sites, and possibly via different mechanisms, and thus assess the overall impact of the chemical on function at the organismal level; and *c*) can develop models that quantitatively describe situations in which endocrine disruptors and reproductive toxicants acting by other mechanisms are both present, since this ultimately represents the real-world scenario.

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